ROBIC

Une société en nom collectif
A General Partnership

DEPUIS 1892 - ESTABLISHED 1892

AGENTS DE BREVETS ET DE MARQUES - PATENT AND TRADEMARK AGENTS
55 St-Jacques, Montréal, Québec, Canada H2Y 3X2
Tél.: (514) 987-6242 Fax : (514) 845-7874
www.robic.ca

November 3rd, 2003

EUROPEAN PATENT OFFICE
PCT DIVISION
Erhardstraβe
D-80331 Munich
GERMANY

VIA FACSIMILE
(confirmation by COURIER)

RE : UNIVERSITE DE MONTREAL et al.
International patent application
No. PCT/CA03/00487 filed on April 3rd, 2003
Our ref.: 000711-0024

Dear Sirs:

A- We hereby refer to the Demand under Article 31 PCT enclosed with this fax and respectfully request that, as International Preliminary Examiner Authority, you proceed to a substantial examination of the above application.

B- We would also like you to take into account the following voluntary amendment made under the provisions of Article 34 PCT.

IN THE DESCRIPTION:

Cancel pages 1, 5, 7, 10, and 12 to 14 of the description presently on file, and insert the new corresponding pages 1, 5, 7, 10 and 12 to 14 enclosed herewith.

IN THE DRAWINGS:

Cancel page 1 of the drawings presently on file, and insert the new corresponding page enclosed herewith.

C- As will be noticed:

- the word "hydrophobicity" has been replaced by --insolubility-- on page 1, line 27 of the description;
- the word "polymerisation" has been replaced by --polycondensation-- on page 2, lines 18 of the description;
- the abbreviation "PGL" has been replaced by --PCL-- on page 5, line 5 of the description;

- the expression "C1-C4" has been replaced by --C_1-C_4-- on page 7, line 6 of the description;
- the word "loose" has been replaced by --lose-- on page 7, line 21 of the description;
- a degree symbol (°) has been inserted after the expression "180" on page 10, line 7 of the description;
- the word "hydrochloride" has been replaced by --acid chloride-- on page 12, lines 14 and 17 of the description;
- the word "chloroforme" has been replaced by --chloroform-- on page 13, line 13 of the description; and
- the expression "palmitoleic acid" has been replaced by --palmitic or oleic acid-- on page 14, line 29 of the description.

The above-mentioned modifications were made to the description essentially to correct obvious typographical and/or clerical errors.

As the Examiner will also note, the chemical formula of the polylactic-co-glycolic acid has been corrected on page 1 of the drawings.

Once again, the above-mentioned modification has been made essentially to correct an error that was noticed in the formula, which error is obvious in view of the name of the compound as given adjacent to the formulae.

D- The Applicant has noted that, in the International Search Report mailed on July 24, 2003, the international application no. WO 03/000766A1 (SHASTRI) (SHASTRI) has been classified in categories X and P.

Designation of category P indicates that inasmuch as this international application no. WO 03/00766 A1 was published on January 3rd, 2003, that is nearly nine (9) months after the priority date of the present application, which is April 5th, 2002, it can be cited against the present application on the ground of novelty only in countries where it will be validated, and never on the ground of obviousness.

The content of this international application No. WO03/00766-A1 is well known to the Applicant. In this connection, it is worth mentioning that this inventor/applicant of this international application No. WO 03/00766A1, namely Mr. Venkatram Prasad SHASTRI, was named as co-inventor when the U.S. application serial No. 60/369,808, the priority of which ahs been claimed herein, was filed on April 5, 2002.

The invention forming the subject matter of the present application is actually directed to a very specific family of polymers that could be considered as encompassed in part within some of very broad definitions given in international application No. WO 03/00766A1 (see for example claim 27), but that are not otherwise disclosed and/or exemplified in the same application.

More specifically, the present application is directed to a very specific family of functionalizable polymers of very specific formula (see claim 1 of the present application). These polymers are prepared by a very simple yet efficient process comprising two basic steps plus an optional third step, which are easy to carry out with high yields (see claim 8). More precisely, the process according to the present invention comprises the steps of:

a) reacting a cyclic ester or diester monomer or cyclic amide or diamide monomer A with an epoxide of formula III (see claim 8) in the presence of a catalyst;
b) subjecting the polymer obtained in step a) to an oxidation to convert the $-CH=CH_2$ group into corresponding CH_2CH_2OH groups; and
c) optionally subjecting the polymer obtained in step b) to another oxidation with a Jones mixture to convert the $-CH_2CH_2OH$ group into corresponding carboxylic groups $-CH_2COOH$.

The oxidation steps b) and/or c) of the above process can be carried out with hydrogen peroxide. However, in accordance with a particularly preferred embodiment of the invention, these steps are preferably carried out under mild oxidation conditions. For example, such oxidation can be carried out hydroboration at low temperature (see claims 10 and 11).

Once the $-CH=CH_2$ groups have been converted into corresponding $-CH_2CH_2OH$ groups, the polymer exhibits several grafting possibilities. As aforesaid, the above-mentioned $-CH_2CH_2OH$ groups can further be oxidized into corresponding $-CH_2COOH$ groups, thereby allowing further grafting possibilities.

As aforesaid, international patent application No. WO 03/00766A1 in the name of SHASTRI discloses a very broad family of polymers prepared from hydroxy acid monomers including particular cyclic ester monomers such as lactones or dioxanones (see paragraph 0096) and from monomers provided with an epoxy function (hereinafter called "epoxides").

The whole description of this international application insists on the fact that the epoxides that are already functionalized. More specifically, this description and the few examples given therein make reference to epoxides that have already been functionalized before being copolymerized with cyclic esters. It is only at a few places, but without any concrete examples and/or further description, that reference is made to the fact that the epoxides could be functionalized after having been polymerized with cyclic esters (see paragraphs 0043, 0036, 0040, 0082 and 0095 and claim 27).

In the description of international patent application No. WO 03/00766A1, reference is also made to the kind of groups that can be used for the functionalization (see for example paragraphs 0069 and 0078). A very broad reference is also made therein made to the fact that one of the functionalizable groups could actually be an unsaturation (see paragraph 0070 and claim 30). However, once again, no concrete examples and/or further description is given.

Thus, international patent application No. WO 03/00766A1 encompasses within its very broad disclosure, at least in part the first step of the process according to the present invention, in terms of its starting compounds and type of reaction to be carried out.

The very broad description of international patent application No. WO 03/00766A1 to SHASTRI could also be considered as encompassing, at least in part, the second step of the process according to the present invention, inasmuch as mention is made therein to the fact that the functionalizable group of the epoxy monomer can actually be an unsaturation.

However, nowhere in this document, there is disclosed or suggested that this second step is and could actually be an oxidation step.

In this connection, it is submitted that it was not obvious at all when the present invention was made that one could proceed to the oxidation of an unsaturation at one end of an epoxide after this epoxide has been polymerized with an hydroxy acid monomer, without interfering with the ester links of the polymer.

The present invention is actually based of the discovery made after numerous research and experiments, that there is no actually no interference between the ester links or the polymer obtained in the first step of the process according to the invention when this polymer is substantively subjected to an oxidation in order to convert the unsaturation of the epoxide into a $-CH_2CH_2OH$ group.

In addition to the above, the Applicant notes that, in international patent application No. WO 03/00766A1, a very broad reference is made to potential uses of the compounds that are prepared as disclosed therein. The main advantage in all cases is that the obtained polymers are actually "degradable" (see in particular paragraph 0037).

Amongst the potential uses of these polymers, reference is made to the preparation of particulate and/or capsules in which an active principle can be dispersed or encapsulated in order to obtain an *in vivo* controlled release (see paragraph 00121 and following). However, there is no disclosure or suggestion in this international patent application that the obtained polymers could be grafted to ligands, lipids, pepsides, etc..., as is disclosed, exemplified and claimed in the present application (see claim 13). In other words, the international patent application to SHASTRI does not make specific reference to the preparation of active vectors. No reference is also made therein to the fact that the particulate of capsules that are obtained may be of a size lower than 1 μ.

As aforesaid, the present invention is "restricted" to a very specific amide polymers, their preparation and their uses, which could be considered as broadly disclosed in international patent application No. WO 03/00766A1 but are not actually disclosed and/or exemplified therein.

It is the Applicant's contention that, almost everywhere throughout the word, it is well settled in Law that any new chemical compound that has been prepared for the very first time and has proved to be actually useful, should be considered as patentable.

In this connection, it is submitted that in chemistry, and more particularly in organic chemistry, most of the new chemical compounds produced today are homologs or analogs of other well known compounds. It is also submitted that most of the processes presently used are actually well known processes that have never been used for preparing the very specific compounds of interest. In all cases, the preparation of a new compound and the discovery of this new compound may be useful may nevertheless be considered as an invention, since, in most cases, it can be held that no "prediction" is possible in chemistry.

From a practical standpoint, any chemical compound could be considered as known *per se* because, in theory, a competent chemist could write a list of all possible chemical compounds that might be brought into existence. However, this theoretical possibility does not prevent the new chemical compound with a given utility from being an invention.

In other words, it is believed that inventors who discover a new chemical compound and are in a position to prove that this compound has been prepared and tested with success for a very specific purpose, should actually be entitled to a patent. In this connection, it is believed that it is immaterial that other chemists may have, at any time before, simply written the formula or broadly disclosed and such could be done, without having actually made it.

Under the present circumstances, it is hereby submitted that the functionalizable polymers forming the subject matter of the present application, the process for their manufacture and their very specific uses as presently claimed should be held patentable, since the polymers according to the invention as recited in claim 1, its process of manufacture and their use are structurally new. Some of the compounds encompass with this formula are actually been prepared and have proved to be useful. The fact these polymers according to the invention are similar to the "compounds belonging to very broad class of compounds like those disclosed in international patent application No. WO 03/00766A1 does not make them necessarily unpatentable.

For the above mentioned reasons and in addition to the fact that international patent application No. WO 03/00766A1 was published after the priority date of the present application, a favourable decision as the patentability of the invention disclosed and claimed in present application is earnestly solicited.

Respectfully submitted

ROBIC

Thierry Orlhac

TO/DD/fm

FUNCTIONALIZED POLYMERS AND THEIR BIOMEDICAL AND PHARMACEUTICAL USES

BACKGROUND OF THE INVENTION

A) Field of the invention

The present invention relates to new functionalized polymers that can be used to prepare functionalized polymers particularly useful in the biomedical and pharmaceutical fields.

The invention also relates to the preparation of these functionalizable polymers.

The invention further relates to the functionalized polymers prepared from said functionalizable polymers.

B) Brief description of the prior art

It is known that some alphahydroxy acid polyesters have been used during the past twenty years in the biomedical and pharmaceutical fields. They are used essentially because of their ability to degrade by hydrolysis into corresponding hydroxy acids which are already present in some metabolic pathways.

Figure 1 identified as "prior art" illustrates three of these known polyesters. In the formulae given in this Figure 1, m and n are selected so that the average molecular weight of the corresponding polyester(s) ranges from 1,000 to 80,000.

If these alphahydroxy acid polyesters in the form of macromolecules (*viz.* PLA, PLGA or PCL) are interesting, they unfortunately have also some undesired properties, such as a high insolubility and a negative zeta potential when used in the form of microparticles or nanoparticles. Such also gives them a high reactivity with respect to the reticulo-endothelial system.

To overcome the above mentioned problems, it has already been suggested to prepare and use polymers having a polymeric backbone with lateral

groups such as hydroxyl groups, on which active molecules can be grafted by a covalent bond (prodrug).

As examples of such functionalizable polymers and their potential uses, reference can made to those described in U.S. patent No. 6,093,792 of 2000 (GROSS et al), which are prepared by reaction between a first comonomer selected among lactones, lactides, lactams, thiolactones and non-functionalized cyclic carbonates, and a second, functionalized cyclic carbonate comonomer to which an active substance such a protein, an anticancer drug or an antihypertensive drug, can be linked.

As other examples of such functionalizable polymers, reference can also be made to the PLA-based polymers referred to in column 1 of the above mentioned U.S. patent No. 6,093,792.

PLA-based polymers having lateral carboxylic groups have already been prepared by copolymerisation with malic acid. Such malic-co-lactic polymers with pendant groups can be grafted to various molecules such as other polymers, lipids, ionisable function or antibodies. However, the preparation of these polymers requires numerous steps, including *inter alia* a necessary protection of the carboxylic groups during condensation. Another drawback is the fact that the number of reticulation bonds that may be obtained by transesterification is difficult to determine.

PLA-based polymers having lateral amino groups have also been prepared by copolymerization with lysine. In addition to requiring numerous steps again, this preparation leads to products containing lysine, which is known to be immunogenic.

SUMMARY OF THE INVENTION

It has now been found that new functionalizable polymers of very interesting structure and properties can be prepared by a very simple process comprising a first and second basic steps plus an optional third step, which are easy to carry out with high yield and thus overcome most of the drawbacks of the

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 identified as "prior art" is a representation of three known alphahydroxy acid polyesters, namely polyactid acid (PLA), polylactic-co-glycolic acid (PLGA) and poly-caprolactone (PCL).

Figure 2 is a schematic representation of a potential use of a polymer according to the invention as a carrier to a ligand specific to Selectine E.

Figure 3 is a schematic representative of the three steps of the process used to prepare the functionalizable polymers disclosed in Example 1, functionalizable.

Figure 4 is the formula of an example of methoxy-PEG based polymer according to the invention.

Figure 5 is the formula of an example of cross-linked PEG-based polymer according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

As aforesaid, the functionalized polymer of formula I according to the invention consists of n units derived from a corresponding number of cyclic ester or diester or cyclic amine or diamine monomers (A), and m units derived from a corresponding number of epoxide monomers (B).

In the above formula I, the number and respective position of the units derived from the monomers (A) and those derived from the monomers (B) may substantially vary. As a matter of fact, they may vary in a random manner and the resulting polymers may be of different structure, like, for example, the following one:

AAABAABAAABBAAABAAAAABBA

As non restrictive examples of cyclic ester or diester monomers (A) usable to prepare the polymers of formula I wherein Z is -O-, reference can be made to:

- dioxanediones of the following formula A1, such as glycolide, dilactide or glycolic lactide;

wherein:

X is a non-functional chain optionally containing one or more heteroatoms but no ester or amide link;

W is - CH_2CH_2OH or -CH_2COOH; and

Y is H, C_1-C_4- alkyl or phenyl;

X and Y being optionally linked to each other as shown in dotted lines.

As non-restrictive examples of epoxide monomers (B) of formula II, reference can be made to the following compounds:

allyl glycidyl ether;

methyl vinyl glycidyl amine;

1,2-epoxy 7-octene;

1-vinyl or alkyl 3,4-epoxy cyclohexane; and

4'-vinyl phenyl glycidyl ether.

In the above formula I, R_1, R_2, n, m and x are advantageously selected so that the average molecular weight of the polymer ranges from 1,000 to 50,000.

In the above formula I, it is also important that the ratio of the number of units derived from monomers (B) to the total of units derived from both the monomers (A) and (B), be ranging from 0.005 to 0.30. In other words, the molar ratio m/x must range from 0.005 to 0.30. If this ratio exceeds 0.30, the obtained polymers may lose most of its advantageous properties.

The functionalizable polymers of formula I can be prepared in a very interesting and efficient manner by the process disclosed hereinabove, which comprises two or three steps depending on whether R_2 has to end with a graftable hydroxy group or a graftable carboxylic group.

The first step comprises mixing together either one or several monomers (A) with one or several epoxide monomers (B). The so prepared mixture is then heated at a temperature higher than 100°C in the presence of a suitable ring opening catalyst. As examples of such catalyst, reference can be made to tin catalyst such tetraphenyl tin, tin hexanoate or tin octanoate. The polymer obtained at the end of this step can then be recovered and purified. Such

compounds are active substances like, for example, a ligand specific to Selectine E which must be delivered to regions wherein selective is expressed (see Figure 2).

EXAMPLE 1

Dilactide and alkyl glycidyl ether were mixed in a round bottom flask with tetraphenyltin as catalyst. The mixture was heated at 180°C for 6 hours. The resulting polymer was dissolved in ethylacetate and purified by precipitation in water.

The double bonds of the polymer were then oxidized to OH by hydroboration and the OH groups were subsequently converted to carboxylic groups by oxidation with a Jones mixture (H_2SO_4, CrO_3 and H_2O).

The above mentioned hydroboration was carried out with BH_3 in tetrahydrofuran at 0°C for 3 h. Then, water, sodium hydroxide and peroxide were added for 30 minutes. The resulting hydroxylated polymer was recovered by extraction with chloroform.

The whole process including the three above mentioned steps is illustrated in Figure 3.

This process was actually repeated several times with different amounts of allyl glycidyl ether. The global yield of polymer was about 75% in each case.

The so prepared polymers were then characterized by gel permeation chromatography (GPC), nuclear magnetic resonance, (NMR) and differential scanning calorimetry (DSC).

Table 1 shows the glass transition temperature Tg of the so prepared polymers, as measured by DSC. Tg values are quite different from PLA which has a Tg of about 50°C. These data proves that despite the fact that the Tg is close to the room temperature, the polymers with 1% and 5% pendant groups can be used to prepare nanospheres and/or microspheres due to their high molecular weight.

EXAMPLE 2

Using substantially the same conditions of reaction as in example 1, functionalizable polymers were also prepared in using caprolactone, butyrolactone, dioxanone and cyclic diglycine as monomers (A).

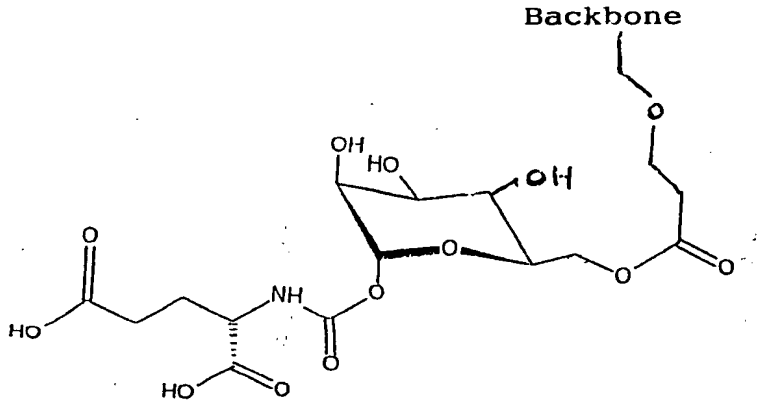
EXAMPLE 3

Some of the functionalizable polymers prepared in Example 1 were used as carriers for a ligand specific to Selectine E. Selectine E is known to be a white cell receptor expressed at the surface of the vascular endothelium in an early stage of adhesion during inflammation.

Grafting of the ligand to the functionalizable polymers was carried out using the following sequence of steps:

- converting the free carboxylic groups of the functionalizable polymer to acid chloride groups;
- protecting all the reactive groups of the ligand;
- selectively unprotecting one of said protected groups of the ligand so that it may react with the acid chloride groups of the functionalizable polymer;
- subjecting the partially unprotected ligand and the functionalizable polymer to esterification; and
- unprotecting all the other reactive groups of the grafted ligand by catalytic hydrogenation.

The obtained functionalized polymer had the following formula:



The average yield of the above process was about 55% and the molecular weights (Mw) of the so-obtained functionalized polymers was 42968 (1% grafting) and 18857 (5% grafting).

Then, microspheres were prepared with an emulsion solvent/evaporation method. To visualize the microspheres and their capability of adhesion to vascular endothelium, these microspheres were labelled with color dyes.

More specifically, batches of microspheres were prepared, containing:

ungrafted polymer, β-carotene (#1);

grafted polymer 5%, Oil Blue N (#2);

grafted polymer 1%, β-carotene (#3).

150 mg of polymers were added to 1.5 ml of a 1% chloroform solution of dye. The organic solution was poured drop wise in 100 ml of a 1% PVA solution under a high shear homogeniser for 3 min. After its formation, the emulsion was subjected to magnetic stirring for 2h to evaporate the organic solvent. Microspheres were collected by centrifugation (5 min, 2000) and washed three times, (yield 87%). Microspheres were dried using a fast freeze dryer.

Mean diameter of microsphere batches were measured by image analysis using Zeiss® optical microscope mounted with a CDD digital camera. Image was grabbed by the Northern Eclipse® acquisition software and analyzed by Optimas 5® image analysis software.

Ex vivo experiments were done on mesenteric rat vessels. Rats were previously treated for three weeks with L-NAM (NO's inhibitor) before *ex vivo* experiment to be in chronic inflammatory condition.

Vessels were removed by surgery and immediately placed in a oxygenated Krebs solution at 37°C.Vessels were opened longitudinally and placed for 5 min into a suspension of microspheres (50% of polymer having ligand, 50% of polymer without ligand) in Krebs oxygenated solution. Tissues were rinsed for 5 min with a clear Krebs oxygenetad solution. Particle count and size measurements were done by optical microscopy and image analysis for each color.

Optical microscopy demonstrated that the microspheres consisting of the polymer with the ligand grafted on it adhered strongly to the endothelium. It also demonstrated that microspheres consisting of polymers without ligand were washed during the process and did not adhere.

Such is a clear indication that the bioadhesive drug carrier that was so prepared, can specifically target Selectine E at the endothelial surface and can therefore be of interest in the treatment of many pathology.

EXAMPLE 4

Amphiphilic PEG-based polymers having the structural properties required for use as stealth drug carriers were also prepared. These carriers may be used to deliver an active substance during several weeks after their intravenous injection. In fact, these carriers differentiate from PEG-ylated liposomes due to their stability (solid matrix) and their covalent bonds.

An example of such an amphiphilic methoxy-PEG-based polymer is illustrated in Figure 4.

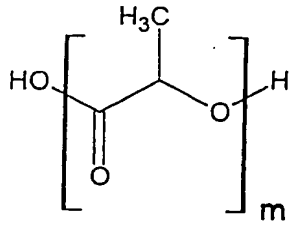
EXAMPLE 5

Other PEG-based polymers having suitable properties for use as a cellular or tissue supports way prepared by grafting PLA to them.

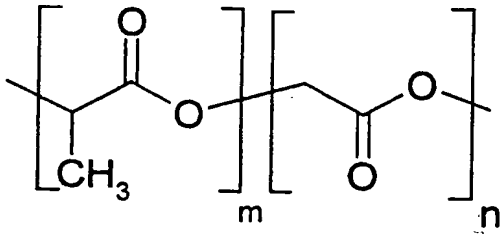
An example of such a polymer is illustrated in Figure 5. It has the advantage of combining the structural features of PLA with the biological features of PEG.

EXAMPLE 6

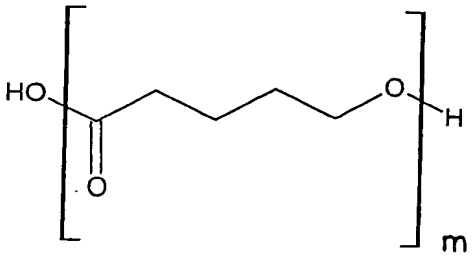
Orally administrable lipids were also prepared. Grafting of these lipids with palmitic or oleic acid was successfully tested. It can be presumed that nanospheres and/or microspheres having correctly chosen lipids on their surface would allow intestinal assimilation.



POLYLACTIC ACID
PLA



POLYLACTIC-CO-
GLYCOLIC ACID
PLGA



POLYCAPROLACTONE
PCL

FIG. 1